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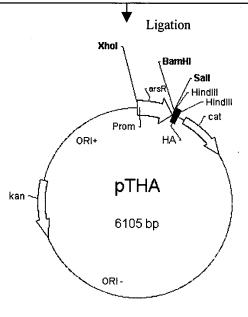
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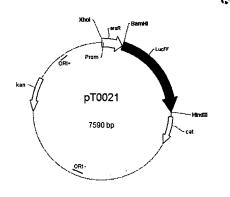
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## FIGURE 1B



#### XhoF-5'-AATTCTCGAGTAAAATAACAT-3

XhoI

AAATCAGGTGACTGTTGAGAAAAAGGAGGCGGATCCCG-BamHNR

PCR of pT0021 with XhoF and BamHNR

Stop of arsR

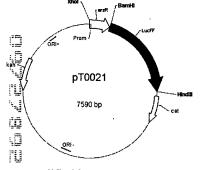
**RBS** 

BamHI

Digestion with Xhol and BamHI

Ligation

#### PCR of pT0021 with LucFFB and LucFFH



LucFFB-5'-CGGGATCCATGAGGGGTTCCGAAGACG

BamHI Start of LucFF Original BamHI

was modified

Modified between stop

of arsR to BamHI

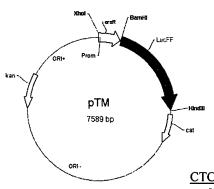
14

-- GAAAGTCCAAATTGTAAGCTTGGG-LucFFH Stop of HindIII

LucFF

Digestion with BamHI and HindIII

Ligation



- Modified in the vicinity of BamHI
- Cloning site for ORFs: BamHI and HindIII
- No additional codons in the induced protein

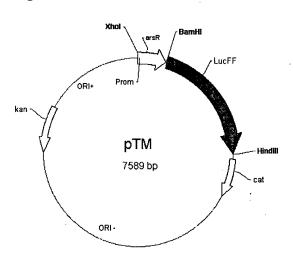
Prom

arsR

Xhol

CTCGAG-----TGAGAAAAGGAGGCGGATCCATG------**RBS BamHI** 

TAAGCTT HindIII

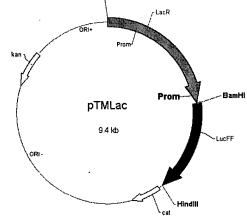


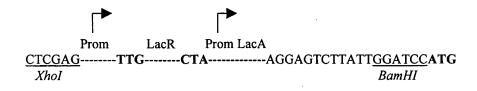
## PCR of Staphylococcus aureus DNA with lacF and LacR

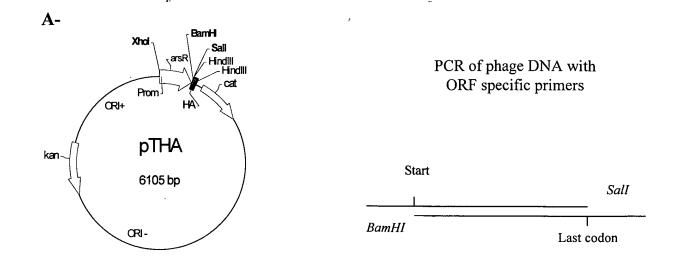
LacF-5`-CCGCTCGAGCTCCAAATTCCAAAACAG
XhoI

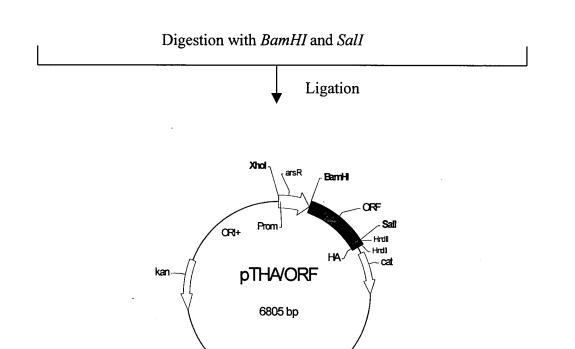
 $\frac{\text{GTAAAAAGGAGTCTTATT}\underline{GGATCC}\text{CG-LacR}}{\textit{BamHI}}$ 

# Digestion with XhoI and BamHI Ligation

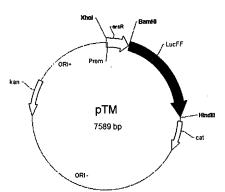






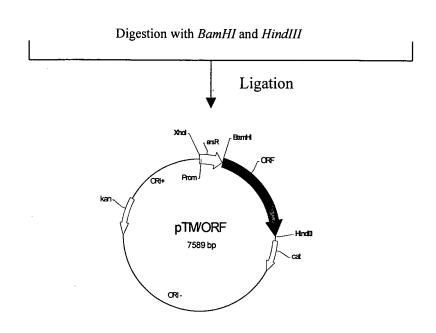


ORI-



PCR of phage DNA with ORF specific primers





#### A) Functional assay on semi-solid support medium

O/N culture inoculated from frozen stock of phage ORF *Staphylococcus aureus* RN4220 transformants

1:10, 1:1000, 1:1000, 1:10000 dilution in saline solution

3 ul of dilution

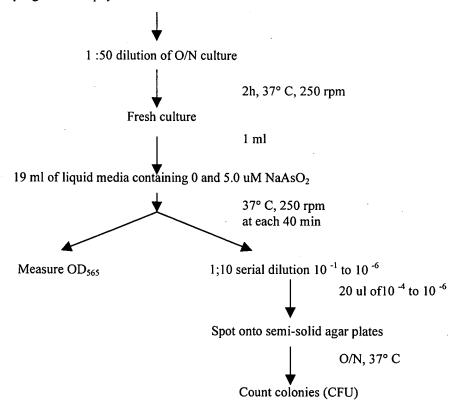
Spot on semi-solid support agar plates containing
0, 2.5, 5.0, 7.5 uM NaAsO<sub>2</sub>

O/N, 37° C

Compare bacterial growth on plates with and without NaAsO<sub>2</sub>

#### B) Functional assay in liquid medium

O/N culture inoculated from frozen stock of phage ORF *Staphylococcus aureus* RN4220 transformants

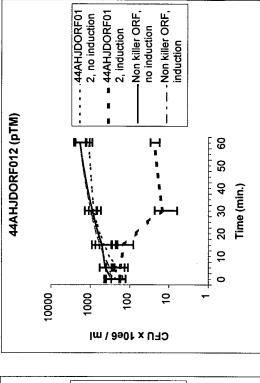


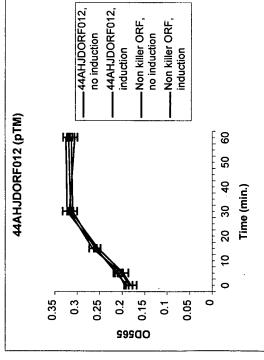
#### 44AHJD ORFs tested for functional analysis

UID	POS
44AHJDORF001	1262710342
44AHJDORF002	37895732
44AHJDORF003	66268389
44AHJDORF004	876410227
44AHJDORF005	1389012643
44AHJDORF006	8032029
44AHJDORF007	20443027
44AHJDORF008	30203775
44AHJDORF009	57446496
44AHJDORF010	1442013938
44AHJDORF011	1559315225
44AHJDORF012	83918813
44AHJDORF013	1499614586
44AHJDORF019	98369630
44AHJDORF023	64946315
44AHJDORF025	1517514999
44AHJDORF027	1291613080
44AHJDORF028	92359071
44AHJDORF035	1395713811
44AHJDORF036	1016510019
44AHJDORF039	17431883
44AHJDORF040	97409877
44AHJDORF044	1291712783
44AHJDORF046	48915019
44AHJDORF048	1534015212
44AHJDORF049	57845909
44AHJDORF053	33483467
44AHJDORF113	199600
44AHJDORF114	1617215870
44AHJDORF121	1636216165
44AHJDORF123	614796

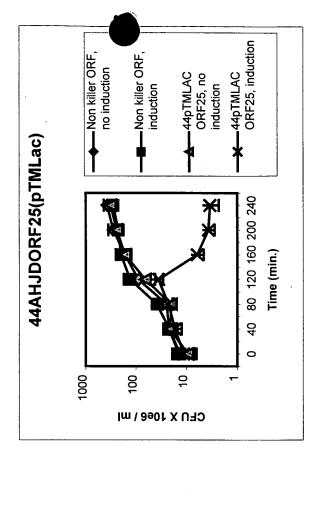
4	Í	

	Semi-solid support media					
ORF ID	Staphylococcus aureus transformants	Without induction	With induction (5 uM sodium arsenite)			
44AHJDORF12	Clone1					
	Clone2	6 9 1				
	Clone3					
44AHJDORF25	Clone1					
	Clone2					
	Clone3					
Control 77ORF30	Clone1		0 0 0			





Non killer ORF, no induction .44pTORF025, No induction ■Non killer ORF, induction -44pTORF025, Induction 44AHJDORF025 (pT) 240 500 120 Time (min.) 40 0.01 0.001 0.0001 0.7 CEU X 10e6 / ml Non killer, no 44pTORF25 No induction 44pTORF25 ► Non killer, induction 44AHJDORF025 (pT) ロコトロミド induction induction 240 160 Time (min.) 80 0 0.4 0.2 0.1



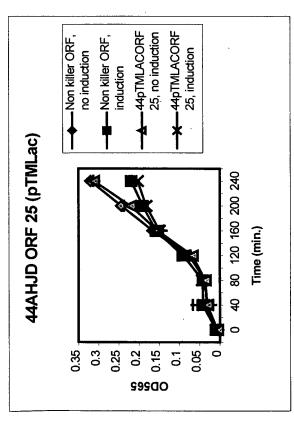
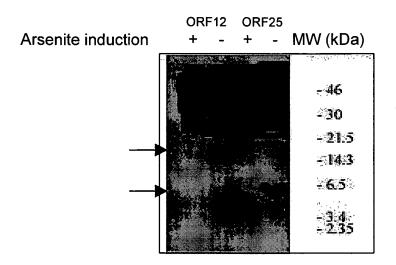


Fig. 5B



### FIGURE 7A

 GST
 GST ORF25

 Mr
 0
 0.1
 0.5
 1.0
 2.0
 0
 0.1
 0.5
 1.0
 2.0
 ACB

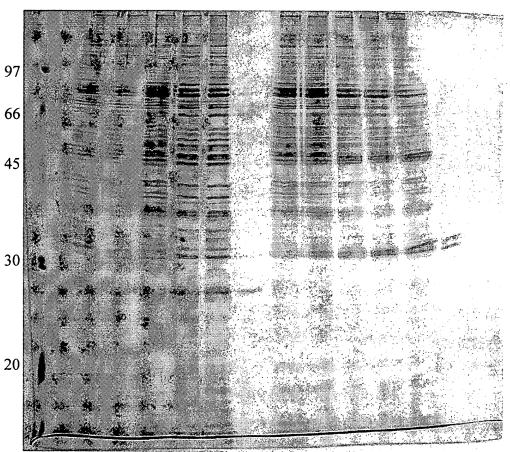
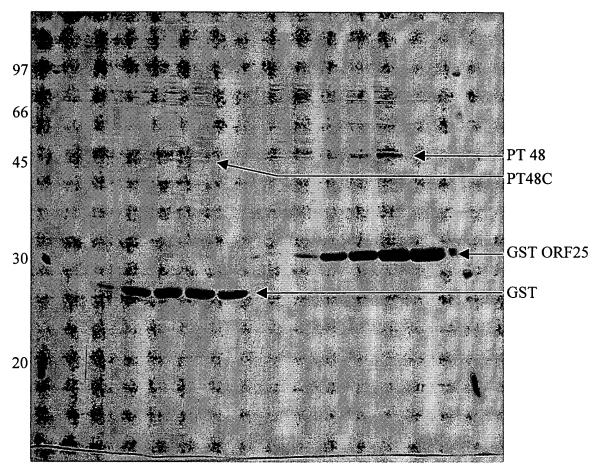


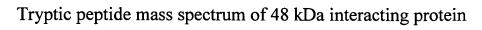
FIGURE 7B

GST ORF25

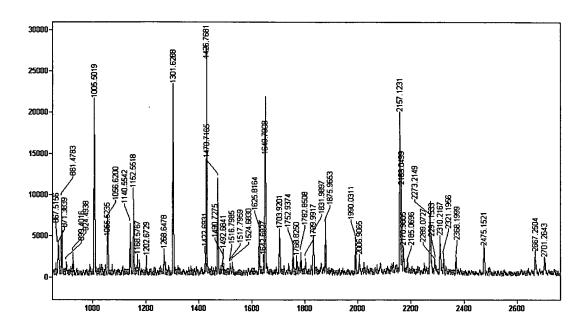
Mr 0 0.1 0.5 1.0 2.0 ACB 0 0.1 0.5 1.0 2.0 ACB Mr





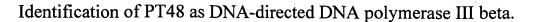


(from Figure 1B)





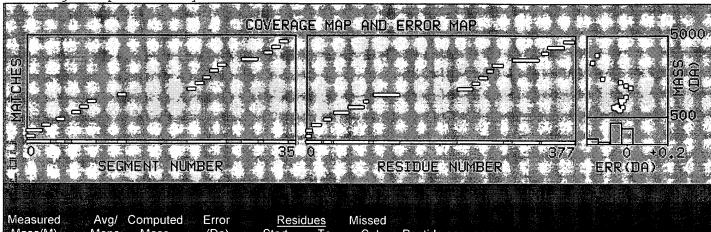




## Details for: PT48 gi|1706496|sp|P50029|DP3B\_STAAU\_DNA\_POLYMERASE III, BETA CHAIN gi|1084187|pir||S54708 DNA-directed DNA polymerase (EC 2.7.7.7) III beta chain - Staphylococcus aureus

Sample ID: PT 48

Number of Measured Peptides : 40 Number of Matched Peptides : 17 Coverage of protein sequence : 68%



Measured	Avg/	Computed	Error	Resi	<u>dues</u>	Missed 🦠	
Mass(M)	Mono	Mass	(Da)	Start	То	Cut	Peptide sequence
	李明					<b>6</b> 1	泰岛自己中央教育的安保中央企业。
870.370	M.	870.397	-0.027	333	339	0	YMMDALK
£880.474					88	0	FFVDIIK
898.392		898.429			7		MMEFTIK: 5
月004.492。			A. C. A. S.	4.000	243	0	VGNVNFISR
1054.514			2001-04-01 - Protect Aug 2000 - 1-10 1994	eration contract the second con-	8	1	MMEFTIKR
그055.603				baddista ka Millara	35		TTLPILTGIK
重151.546					263	200 200 274	LEPENYEIK
1300.619					OH 100 - MINE OF		LLEGHYPDTTR
1425.769					375		GDDSVTQLTLPIR
1469.713							DYFITQLNDTLK
1648.781					277	See 1992 1993	ESTONGEFYHAIDR
2156.112							TVDGEDIVNISETGSVVLPGR
2309.198						0	EHEVILTGSDSEISTEITIPK)
2474.139					234	000000	IMSDNEEDIDIFFASNOVLFK.
2666.266	200		25 - 200 CO. CO.	300			AIDNDEVEVEFFGTMKPFTLKPK
3577670 4033.298					199	999 - Carrier 1	LSTGDDVVELSSTSPEIGTVKEEVDANDVEGGSLK
4000.296	A	4033.421	-U.1Z3	. 97	T32	U	LSTNEOFOTLITSGHSEFNLSGLDPDOYPLLPOVSR

 $\backslash$ 





#### FIGURE 10

SEQ ID NO: 1 Staphyloccus aureus DnaN nucleotide sequence

ATGATGGAATTCACTATTAAAAGAGATTATTTTATTACACAATTAAATGACACATTAAAA GCTATTTCACCAAGAACAACATTACCTATATTAACTGGTATCAAAATCGATGCGAAAGAA CATGAAGTTATATTAACTGGTTCAGACTCTGAAATTTCAATAGAAATCACTATTCCTAAA ACTGTAGATGGCGAAGATATTGTCAATATTTCAGAAACAGGCTCAGTAGTACTTCCTGGA CGATTCTTTGTTGATATTATAAAAAAATTACCTGGTAAAGATGTTAAATTATCTACAAAT GAACAATTCCAGACATTAATTACATCAGGTCATTCTGAATTTAAGTTGAGTTAGAT CCAGATCAATATCCTTTATTACCTCAAGTTTCTAGAGATGACGCAATTCAATTGTCGGTA AAAGTGCTTAAAAACGTGATTGCACAAACAAATTTTGCAGTGTCCACCTCAGAAACACGC CCAGTACTAACTGGTGTGAACTGGCTTATACAAGAAAATGAATTAATATGCACAGCGACT GACTCACACCGCTTGGCTGTAAGAAAGTTGCAGTTAGAAGATGTTTCTGAAAACAAAAAT GTCATCATTCCAGGTAAGGCTTTAGCTGAATTAAATAAAATTATGTCTGACAATGAAGAA ATTTCTCGATTATTAGAAGGACATTATCCTGATACAACACGTTTATTCCCTGAAAACTAT GAAATTAAATTAAGTATAGACAATGGGGAGTTTTATCATGCGATTGATCGTGCCTCTTTA TTAGCGCGTGAAGGTGGTAATAACGTTATTAAATTAAGTACAGGTGATGACGTTGTTGAA TTGTCTTCTACATCACCAGAAATTGGTACTGTAAAAGAAGAAGTTGATGCAAACGATGTT ATCGATAATGATGAGGTTGAAGTTGAATTCTTCGGTACAATGAAACCATTTATTCTAAAA CCAAAAGGTGACGACTCGGTAACGCAATTAATTTTACCAATCAGAACTTACTAA

#### SEQ ID NO: 2 Staphyloccus aureus DnaN amino acid sequence

>gi|1084187|pir||S54708 DNA-directed DNA polymerase (EC 2.7.7.7) III beta chain - Staphylococcus aureus

MMEFTIKRDYFITQLNDTLKAISPRTTLPILTGIKIDAKEHEVILTGSDSEISIEITIPKTVDGEDIVNI SETGSVVLPGRFFVDIIKKLPGKDVKLSTNEQFQTLITSGHSEFNLSGLDPDQYPLLPQVSRDDAIQLSV KVLKNVIAQTNFAVSTSETRPVLTGVNWLIQENELICTATDSHRLAVRKLQLEDVSENKNVIIPGKALAE LNKIMSDNEEDIDIFFASNQVLFKVGNVNFISRLLEGHYPDTTRLFPENYEIKLSIDNGEFYHAIDRASL LAREGGNNVIKLSTGDDVVELSSTSPEIGTVKEEVDANDVEGGSLKISFNSKYMMDALKAIDNDEVEVEF FGTMKPFILKPKGDDSVTQLILPIRTY